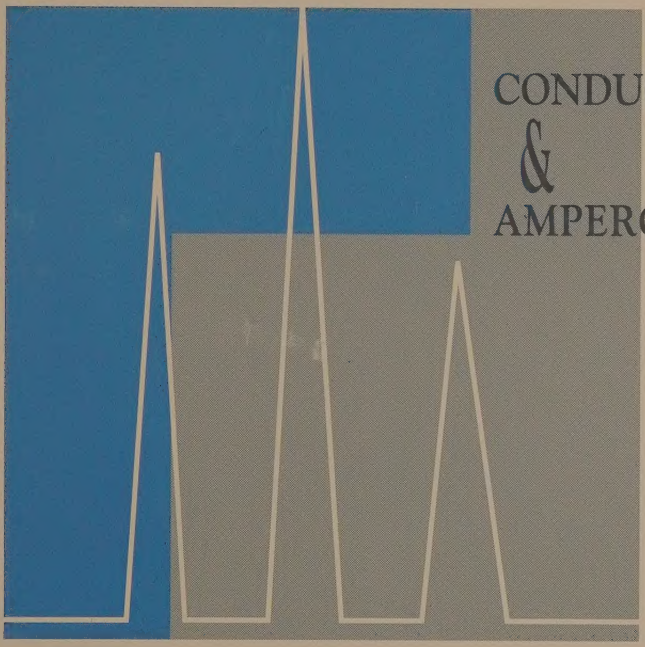

CONDUCTIVITY & AMPEROMETRY

Electrochemical Detection in Liquid and Ion Chromatography

ROY D. ROCKLIN

DIONEX

Conductivity and Amperometry

Electrochemical Detection in Liquid and Ion Chromatography

by

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Introduction

Electrochemical detectors measure current resulting from the application of potential (voltage) across electrodes in a flow cell. Depending on the method by which the potential is applied and the current measured, several different properties of the flowing solution can be determined. These measurements help answer the major questions analytical chemists ask: What's in it and how much?

Of course, there are other detectors used in High Performance Liquid Chromatography. In the years since HPLC became a powerful analytical technique, the UV-visible absorbance detector has dominated all other forms of detection. Sensitivity is excellent for many analytes, especially aromatic species, while transparent mobile phases are readily available. However, there are numerous analytes that have very poor absorbance and are not detected with sufficient sensitivity. Most of these nonchromophoric molecules are organic aliphatics and inorganic ions. Low wavelength UV occasionally can be used, but at a loss in selectivity. Refractive index detection is often used. However, RI detection is less sensitive than UV and substantially less selective.

Fortunately, a wide variety of nonchromophoric molecules can be detected with good or excellent sensitivity by one of several forms of electrochemical detection. These are carboxylic, sulfonic, and phosphonic acids; alcohols, glycols, and aldehydes; carbohydrates; primary, secondary, tertiary, and quaternary amines; sulfates, sulfoxides, thiols, sulfides, and mercaptans; and inorganic anions and cations.

This book presents the fundamentals of electrochemical detection methods. It provides you with an understanding of how you can use these powerful techniques to determine analytes from the many categories listed above.

Electrochemical Detection Methods

There are three major forms of electrochemical detection: conductivity, amperometry, and integrated amperometry. (Pulsed amperometry is a form of integrated amperometry.) All three of these methods are performed on the Dionex Pulsed Electrochemical Detector (PED). In this book, references will be made to the use of this detector. The Dionex Conductivity Detector Module (CDM) is only used for conductivity detection. The Dionex Pulsed Amperometric Detector (PAD) can be used to perform amperometry and pulsed amperometry, but not integrated amperometry.

During conductivity detection, analytes move in the electric field between electrodes in the cell. At any instant in time, negatively charged anions migrate toward the positive electrode and positively charged cations migrate toward the negative electrode. The conductivity of a solution is directly proportional to the mobility of the ions in solution. No oxidation or reduction reactions take place, since there are no electron transfer reactions.

For all forms of amperometry, current (or charge) resulting from the oxidation or reduction of analyte molecules is measured. During an oxidation reaction, electrons are transfered from the analyte to the electrode. During a reduction reaction, the reverse occurs. Electrons leave the electrode and enter the analyte. This concept is illustrated in Figure 1.

Equivalent Circuits

The Pulsed Electrochemical Detector measures current resulting from the application of potential (voltage) across electrodes in a flow cell. The three forms of electrochemical detection differ in the manner used to apply the potential and measure the current.

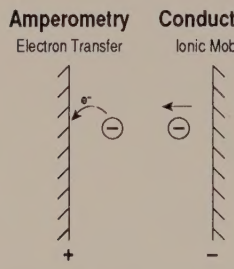
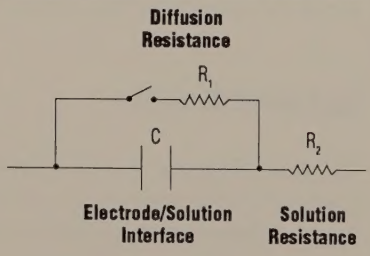


Figure 1 Properties measured during electrochemical detection. Amperometry — current (or charge) from electrons transferred between neutral or ionic analytes and the electrode. Conductivity — mobility of ions in an electric field.

To understand how the PED can measure these very different quantities simply by applying voltage and measuring current, it is useful to model electrochemical cells in the equivalent circuit shown in Figure 2. This circuit is designed to mimic the electrochemical behavior of conductivity and amperometry cells. R_2 represents the resistance to the migration of ions caused by the electric field applied between the electrodes, measured during conductivity detection. The interface between the solution and the electrode acts as a capacitor (C in the circuit). When the applied potential is high enough, electrons transfer to or from species in solution. This is represented by the closing of the switch. The resulting current measured during amperometry is controlled mostly by the rate of diffusion of analyte to the electrode surface. A resistance (R_1) may be defined which is inversely proportional to the rate of analyte diffusion to the electrode surface.

Conductivity. The quantity we wish to measure during conductivity detection is the inverse of the solution resistance R_2. It can only be measured when all of the applied potential develops across R_2. This is

Figure 2 Equivalent circuit used to model a working electrode in solution. Closing the switch represents the onset of electron transfer (oxidation/reduction) reactions measured during amperometry. R_1 represents a resistance inversely proportional to the rate of diffusion of analyte molecules to the surface of the working electrode. R_2 represents the resistance to migration of analyte molecules in the electric field between the electrodes, measured during conductivity detection.



accomplished using a high frequency alternating potential applied across the electrodes. When an alternating potential of several kHz is applied across a capacitor, its resistance to current flow drops to near zero. With zero resistance (more accurately, reactance) at the interface between the electrode and the solution, all of the applied potential develops across the solution resistance R_2. Since we know the applied potential and the current is measured, R_2 is calculated from Ohm's law, $R = E/I$. The inverse of the solution resistance R_2 is the *conductance*. The measured conductance is then corrected by the conductivity cell constant to produce the conductance which would be measured in a cell containing electrodes of 1 cm^2 surface area held 1 cm apart. This quantity is the *conductivity*, and the units are siemens per cm, S/cm.

Amperometry. The addition of a supporting electrolyte in high concentration greatly lowers the solution resistance. (An *electrolyte* is simply a solution containing dissolved ions.) Generally, acids, bases or salts in concentrations of 1 to 100 mM are used. The addition of a supporting electrolyte plus the application of a d.c. or very low frequency alternating potential waveform causes nearly all of the applied potential to develop across the interface between the electrode and the solution. The potential difference between the electrode and the solution can easily be made high enough to cause electron transfer reactions to occur (closing the switch), oxidizing or reducing species in solution.

During amperometry, the current is reported directly to the recording device. With the other methods (conductivity, integrated and pulsed amperometry) the detector converts the measured current into other, proportional, units. The detection methods and the three properties of the solution which are measured are listed below.

Method	Quantity Measured	Units
Conductivity	Solution conductivity	siemens (S)
Amperometry	Current caused by reduction or oxidation of solution species	amperes (A)
Integrated and Pulsed Amperometry	Integrated current (charge) from reduction or oxidation of solution species during a portion of a repeating potential vs. time program	coulombs (C)

In summary, the detection method is determined by the applied potential waveform, whether or not a supporting electrolyte is added, and the manner in which the current is measured.

Conductivity Detection

Species Detected by Conductivity

Conductivity detection with liquid chromatography has been used for years to determine inorganic ions. The technique is called *ion chromatography.* By one of the more popular definitions, ion chromatography is simply the chromatography of ions. Since ions are both inorganic and organic, the same technique can be used for organic ions as well. Whether the analytes are organic or inorganic, conductivity is typically the detector of choice for species which are ionic when they enter the detector cell. This includes all anions and cations of strong acids and bases, such as chloride, sulfate, trifluoroacetate, sodium, and potassium. Ions of weaker acids are detected provided that the mobile phase pH is chosen to maximize analyte dissociation. (When a suppressor is used, the mobile phase pH which determines whether or not an ion will be detected is the pH after suppression.) As analyte ionization (dissociation) decreases, so does sensitivity. For anion exchange chromatography, sensitivity is poor for anions with pK_a's above 6. Anions with pK_a's above 7 are not detected. Fortunately, all organic acids with either carboxylate, sulfonate, or phosphonate functional groups have pK_a's below 4.75, so conductivity is often the prefered detection method for these species.

Nearly all organic cations are amines. Aliphatic amines have pK_a's around 10 and are easily detected. Aromatic and heterocyclic amines have pK_a's between 2 and 7, too low to be detected by suppressed conductivity following cation exchange separation. Although nonsuppressed (single column) detection can be used for these species, sensitivity is generally poor. These amines can be detected by UV absorbance or by d.c. or integrated amperometry.

Zwitterionic molecules can be difficult or impossible to detect by conductivity. These molecules contain both cationic and anionic functional groups. Amino acids are good examples. They contain both ammonium

cationic functional groups and carboxylic acid anionic functional groups. With suppressed conductivity detection, they are generally removed by the suppressor and do not reach the detector. Using non-suppressed conductivity detection, they can be detected provided the mobile phase pH is at a value which results in a net charge on the molecule. Since most zwitterions contain primary, secondary, or tertiary amine functional groups, pulsed or integrated amperometry is often the best detection method. UV absorbance can be used for aromatic zwitterions.

Conductivity of Solutions

To understand conductivity detection, it is useful to to review how individual ions contribute to the total conductivity of solutions.

The conductivity of a dilute solution is the sum of the individual contributions to conductivity of all the ions in the solution multiplied by their concentrations; i.e. conductivity is directly proportional concentration. This is called Kohlraush's law of independent migration. It states that each ion carries its portion of the total conductivity without being affected by any of the other ions in solution. Stated as an equation:

$$\kappa = \frac{\Sigma_i \lambda^\circ_i c_i}{1{,}000}$$

where κ is the measured conductivity in S/cm, and c_i is the concentration of the ions in equiv/L. (Equiv/L equals moles/L times the charge on the ion.) λ°_i, the *ionic limiting equivalent conductivity*, is specific for each ion. It is the conductivity of the ion divided by concentration and extrapolated to infinite dilution. Table I lists limiting equivalent conductivities for a number of organic and inorganic ions in aqueous solution at 25°C. The unit for λ°_i is S•cm²/equiv.

Values of λ°_i from Table I can be used to calculate the conductivities of solutions containing ions. For example, the limiting equivalent conductivity at 25°C for NaCl is the sum of the ionic limiting equivalent conductivity for Na^+, 50.1, plus that of Cl^-, 76.4, or 126.5. A 0.1 mM solution of NaCl at 25°C will have a conductivity of 0.1 x 126.5, or 12.65 µS/cm. The

Table I
Equivalent Conductivities

Anions	λ°_i	Cations	λ°_i
OH^-	198	H^+	350
F^-	54	Li^+	39
Cl^-	76	Na^+	50
Br^-	78	K^+	74
I^-	77	NH_4^+	73
NO_3^-	71	Mg^{2+}	53
HCO_3^-	45	Ca^{2+}	60
SO_4^{2-}	80	Sr^{2+}	59
$Acetate^-$	41	$CH_3NH_3^+$	58
$Benzoate^-$	32	$N(CH_3CH_2)_4^+$	33

conductivity of a solution containing 0.1 mM NaCl plus 0.1 mM Na_2SO_4 would be:

Ions		Charge		Conc.		λ°_i			
3	x	1	x	0.1	x	50.1	=	15.0	(Na^+)
1	x	1	x	0.1	x	76.4	=	7.6	(Cl^-)
1	x	2	x	0.1	x	80.0	=	16.0	(SO_4^{2-})
								38.6	µS/cm

So far, only dilute solutions have been discussed. As concentration increases, the direct proportionality between conductivity and concentration is lost. However, at the analyte concentrations normally encountered in ion chromatography (below 1 millimolar), conductivity is generally proportional to concentration. For example, the equivalent conductivity at 25°C of KCl at infinite dilution is 149.9; and at 1 mM, 146.9, a decrease of only 2%. (The conductivity of an eluting analyte cannot be assumed to be directly proportional to concentration, because ionic components of the mobile phase may be contained in the eluting volume.)

If the electrolyte is a weak electrolyte such as an acid or base with only partial dissociation, then c_i must be replaced by the concentration of the

dissociated ions only, since only they contribute to conductivity. For acids and bases, the pK values and the solution pH can be used to calculate the extent of dissociation.

Effect of Solvent on Conductivity

The limiting equivalent conductivity of an ion, λ°_i, is a measure of the mobility of the ion. Ionic mobility is greatly affected by the properties of the ion in the solvent. Ions with large hydration spheres are less mobile, and therefore less conductive, than ions with small hydration spheres. This explains why λ°_i for extensively hydrated fluoride (55.4) is lower than λ°_i for chloride (76.4), which is less hydrated. The viscosity of the solvent also affects ionic mobility, with ions being more mobile in solvents of lower viscosity.

It is not necessary to know values such as hydration sphere and viscosity, since quantitative analysis is performed by comparing the conductivity of the analyte in the sample to the conductivity of the same analyte in a standard (or standards). Even if a solvent gradient is used, the composition of the solvent during the elution of the analyte will be the same in both the sample and the standard.

Effect of Temperature on Conductivity

Mobility, and therefore conductivity, is greatly affected by temperature. The conductivity of an aqueous solution is found experimentally to rise about 2% per °C. (This dependence is described in a complex equation developed by Onsager.) Therefore, it is necessary to measure the conductivity independent of temperature, or errors in concentration measurements could result as the mobile phase temperature changes. The PED accomplishes this by correcting the measured conductivity to that which would be measured at 25°C by measuring the cell temperature with a thermistor and multiplying the conductivity by a temperature dependent constant. This constant is called the *temperature compensation factor*, and is expressed in units of percent per °C.

Chemical Suppression

Species detected by conductivity are by their nature ionic, so ion-exchange and ion-pair chromatography are by far the most commonly used separation methods. These methods both require mobile phases containing strong electrolytes. This causes a problem: how to detect the ionic analytes without the detector being overwhelmed by the ions in the mobile phase. The best method of solving this problem is by neutralizing the mobile phase in a suppressor.

The mechanism of suppression, using sodium hydroxide as the mobile phase for anion-exchange separation, is illustrated in Figure 3. Analyte anions elute from the column with sodium counterions. They enter the suppressor between two cation-exchange membranes. On the other side of these membranes a dilute solution of sulfuric acid (the regenerant) flows in the opposite direction. The membranes have fixed negative sulfonic acid functional groups and therefore exclude anions. Positive ions can pass through the membrane freely. Therefore, sodium ions from the mobile phase flow across the membranes into the regenerant side. An equal number of hydrogen ions from the regenerant flow across the membrane and enter the mobile phase side, thus neutralizing the highly conductive hydroxide to water. The resulting background conductivity is near zero, considerably lower than before suppression. Also, the counterion to the

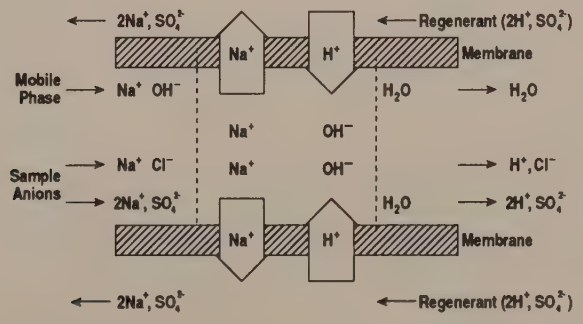


Figure 3 Schematic of chemical suppression (anion exchange). Sodium counterions from the eluant cross the cation-exchange membranes and are replaced by hydrogen ions from the sulfuric acid regenerant, neutralizing the highly conductive hydroxide to water and greatly increasing the signal-to-noise ratio.

anion analytes is now hydrogen ion, which has seven times higher conductivity than the original sodium counterion. Since both the anion analyte and the cation counterion produce the response at the detector, the response is increased. The suppressor both lowers the background conductivity (and therefore the baseline noise and drift) and increases the signal.

For cation ion chromatography, the suppressor membranes are anion exchange polymers. They allow anions to pass freely but exclude cations. Dilute acids such as hydrochloric acid are used in the mobile phase. In the suppressor, the chloride counterions are replaced by hydroxide, neutralizing the acidic mobile phase and providing the highly conductive hydroxide counterion to the analyte cations. Tetrabutylammonium hydroxide is used as the regenerant because it is excluded from the anion exchange membranes.

Advantages the Suppressor Provides. The use of a suppressor typically increases signal-to-noise ratios for strong acid or base ions about one order of magnitude. The improvement is somewhat less for ions of weak acids or bases due to decreased ionization at the neutral pH of the mobile phase following suppression. Nonetheless, the benefits in reduction of the background, and therefore the noise, almost always yield a net improvement in signal-to-noise ratio as compared to nonsuppressed conductivity detection.

The use of a suppressor provides several important advantages. The first three in the list below are a direct result of the increased signal-to-noise ratio.

- Lower detection limits
- Dirty samples may be diluted more, extending column life
- Wider dynamic range
- More concentrated mobile phases can be used, providing a greater range of elution control, and the ability to use larger sample volumes
- Gradient elution capability
- Faster equilibration time
- Elimination of interference from counterions
- Elimination of system peaks

Suppressed ion chromatography requires the addition of a suppressor and a reservoir of regenerant to the analytical instrumentation. Although this increases the complexity of the instrumentation somewhat, continuous recycling of regenerant through a large ion exchange column (provided by the Dionex AutoRegen Accessory) allows the suppression system to be used unattended for weeks.

Mobile Phases for Conductivity Detection

When choosing a mobile phase, the constraints placed by both the separation and detection methods must be considered. To obtain optimum separation, the elution strength of the mobile phase, the separation efficiency, and the resolution of the analytes of interest are the major criteria. For conductivity detection, the criteria for a good mobile phase are the relative conductivity response of the analytes and the magnitude of the background.

Mobile phases can be wholly aqueous containing only water and a strong electrolyte. Or, if an organic solvent compatible column such as a Dionex OmniPac or MPIC column is used, typical reversed phase solvents such as methanol or acetonitrile can be used. Organic solvents are essential components of ion-pair mobile phases and provide important selectivity control during ion exchange separations. Since these solvents are nonconducting, they do not interfere with conductivity detection.

Anion Exchange. When a suppressor is used, the ionic components of the mobile phase must be such that they are removed or converted to weakly conducting compounds by the suppressor. Sodium salts of weak acids are used because they are converted to the neutral free acid form in the suppressor. The higher the acid's pK_a, the lower the background conductivity following suppression. Weak acids with pK_a's above 6 can be used for isocratic separations. However, for gradient elution, pK_a's should be above 8 to minimize baseline shift during the gradient.

Sodium hydroxide solutions make excellent mobile phases for anion exchange because hydroxide is neutralized in the suppressor to water (the free acid form of hydroxide). This is the case regardless of its concentration,

making it most useful for gradient elution. An example of the utility of hydroxide based mobile phases for the separation of both organic and inorganic anions is shown in Figure 4.

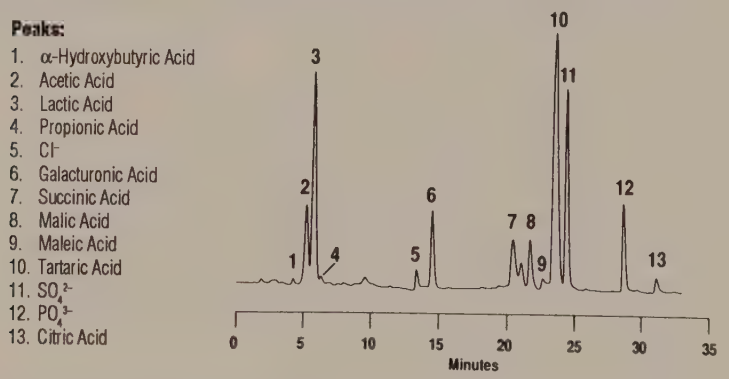


Figure 4 Separation of anions in red wine. OmniPac PAX-500 column with suppressed conductivity detection. Gradient of 1 to 90 mM NaOH with 32% methanol.

Another commonly used mobile phase is a carbonate/bicarbonate buffer. It is suppressed to carbonic acid ($pK_a = 6.2$) which has conductivity low enough for isocratic but not for gradient elution. Carbonate/bicarbonate buffers are easily prepared and are routinely used for isocratic separations of inorganic anions.

Other mobile phases which may be used in suppressed anion chromatography are the sodium salts of boric acid ($pK_a = 9.2$) and p-cyanophenol ($pK_a = 8.0$). Mobile phases containing boric acid are effective at separating very weakly retained anions such as monoprotic carboxylic acids. Borate also forms weak bonds with hydroxy-organic acids, producing changes in selectivity as compared to hydroxide. Because of its high pK_a, background conductivity following suppression is very low, making it useful for gradient elution. p-Cyanophenol is a powerful monovalent displacer, useful for eluting strongly retained hydrophobic anions such as fumaric acid, iodide, and thiocyanate.

In nonsuppressed (often called single column) ion chromatography, the ionic components in the mobile phase are chosen such that their conductivity is as different as possible from the analyte's. For anion IC, large ions with poor mobility such as benzoate and phthalate are often used. These produce backgrounds of moderately high conductivity. A buffer composed of a gluconate borate solution produces somewhat lower backgrounds. Sensitivity and baseline noise are acceptable for medium to high level analyses. However, the advantages provided by a suppresser are not realized.

Cation Exchange. A millimolar concentration of a dilute acid such as hydrochloric acid is a good choice for cation exchange chromatography of the monovalent cations: alkali metals and monovalent amines. However, hydrogen ion is too weak to elute divalent cations such as alkaline earth ions and divalent amines. These more strongly retained cations are eluted using 2,3-diaminopropionic acid (DAP, shown in Figure 5). The ionization constants of the acid are 1.3, 6.7, and 9.4. At pH 1.3 (47 mM HCl) one half of this acid is in the fully protonated divalent form, and one half in the partially protonated monovalent form. The divalent form has much stronger displacing power, making this a good mobile phase component for the elution of divalent cations. The membrane suppressor converts it to its zwitterionic form, which has no conductivity. Using a gradient of HCl, DAP, and acetonitrile, a large number of both amines and inorganic cations can be eluted in a single run (Figure 6).

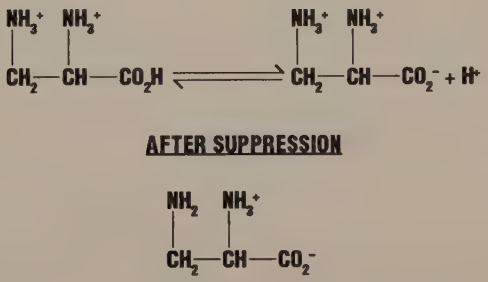


Figure 5 Forms of 2,3-diaminopropionic acid (DAP). K_a for the singly charged to doubly charged forms is 0.047 (pK_a = 1.3). The doubly charged form is an effective eluting agent for divalent cations. After suppression, the neutral zwitterion is nonconductive.

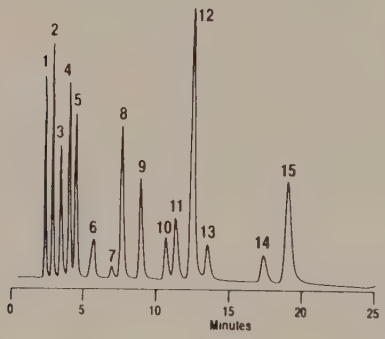


Figure 6 Organic amines and inorganic cations separated on an experimental OmniPac cation-exchange column. Gradient of 20 to 80 mM HCl, 2 to 5 mM DAP, and 6% to 25% CH_3CN.

The best mobile phases for nonsuppressed cation IC are dilute strong acids such as 1 mM nitric acid. Backgrounds are very high (around 1 mS), so signal-to-noise ratios are not good enough for low level work. Since the conductivity of hydrogen ion is greater than that of all other cations, elution of analytes causes decreases in conductivity. Instead of peaks, eluting analytes produce dips.

Ion-Pair. Mobile phases for ion-pair chromatography contain mixes of aqueous and organic solvent solutions with hydrophobic ion-pair reagents as additives. Commonly used reagents are quaternary ammonium salts for anion separations and long-chain sulfonates for cation separations. For suppressed conductivity detection, these reagents are easily used provided the counterions are either hydroxide (anion ion-pair) or hydrogen ion (cation ion-pair). These are provided by Dionex as IonSep reagents. They are purified solutions of quaternary ammonium hydroxide solutions and sulfonic acid solutions.

Amperometric Detection

Amperometric detection is used to measure the current or charge resulting from oxidation or reduction of analyte molecules at the surface of a working electrode. During oxidation reactions, electrons are transfered from molecules of an electroactive analyte to the working electrode in the amperometry cell. Conversely, during reduction reactions, electrons are transferred from the working electrode to the analyte. For analytes which can be oxidized or reduced, detection is usually sensitive and highly selective for electroactive species, since many potentially interfering species cannot be oxidized or reduced, and are not detected. When a single potential is applied to the working electrode, the detection method is *d.c. amperometry*. *Pulsed amperometry* and *integrated amperometry* employ a repeating sequence of potentials.

Voltammetry

The determination of the optimum potentials to use in amperometry begins with an electrochemical technique called *hydrodynamic voltammetry*. This technique is performed by continuously moving a solution containing the analyte and supporting electrolyte past the surface of the working electrode in an amperometry cell. This can be accomplished in a standard beaker-type cell employing a rotated disk electrode, or in the PED amperometry cell with the mobile phase and analyte flowing through the cell. The current as a result of oxidation or reduction reactions is measured and plotted vs. applied potential, which is scanned between preset limits. If the potential is scanned in one direction and then reversed such that the potential at the end of the scan is the same as the beginning, the technique is called *cyclic voltammetry*. Cyclic voltammetry is performed on the PED by programming a triangle wave in the Amperometry mode. The voltammogram is recorded using an X-Y recorder, storage oscilloscope, or computer.

Consider the following generalized electrochemical reaction where, for example, species A could represent ferrocyanide ion and species B, ferricyanide.

$$A \quad B + ne^-$$

When the reaction proceeds in the forward direction, species A transfers n electrons to the working electrode and is oxidized to species B. The reverse reaction is the reduction of B back to A. The direction of the reaction can be predicted by the Nernst equation

$$E_{app} = E^\circ + \frac{0.059}{n} \log \frac{[B]}{[A]}$$

where E_{app} is the applied potential, [A] and [B] are the equilibrium concentrations of species A and B *at the surface of the working electrode*, and E° is the potential at which these two concentrations are equal. (For simplicity, concentrations are used instead of activities.) Each oxidation/reduction reaction has a characteristic E°. Many chemical reference books contain tables of E°'s for various substances listed as reductions versus an H_2/H^+ reference electrode. When $E_{app} = E^\circ$, log [B]/[A] must equal zero; thus [B] = [A]. If E_{app} is set positive of E°, then log [B]/[A] must be greater than zero and therefore [B] > [A]. The reaction proceeds in the forward direction with species A being oxidized to species B. Conversely, setting E_{app} negative of E° results in the reduction of B to A, generating a negative current.

Consider a situation in which only species A is present in solution and where E° is +0.4 volts versus an Ag/AgCl reference electrode. Figure 7 shows the current as a function of applied potential, the *voltammogram*. The shape of the voltammogram can be explained by considering the concentration of A at the electrode surface at three different applied potentials. At 0.2 volts, E_{app} is negative of E°. The Nernst equation indicates that at 0.2V, the reduction of B to A is favored. Because the solution contains only substance A, no reaction occurs. As the potential is increased, it approaches a level high enough to cause oxidation of a percentage of analyte molecules, and the current increases. At a higher potential, 100% of the analyte molecules reaching the surface of the working electrode are oxidized, and the current is no longer dependent on potential. Since the current is now limited by the rate at which the analyte molecules are

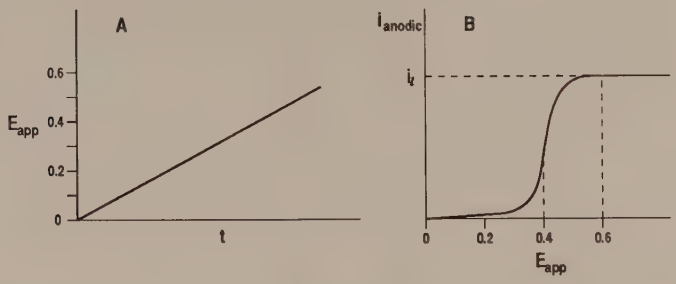


Figure 7 Applied potentials versus time (A) and the resulting voltammogram (B) for hydrodynamic (stirred solution) voltammetry.

transported to the surface of the working electrode, and since this rate is largely dependent on diffusion, this maximum current is called the diffusion limited current.

d.c. Amperometry

Species Detected by d.c. Amperometry. The major application of d.c. amperometry is the detection of catecholamines. These molecules are neurotransmitters, and are also used as pharmaceuticals. There are many other applications of electrochemical detection based on oxidation or reduction. Table II lists the species most commonly determined and the working electrode materials and applied potentials used. The main categories are phenols (including catecholamines), aromatic amines, thiols, and inorganic anions forming complexes with silver, such as cyanide, sulfide, and iodide.

Choosing the Applied Potential. The optimum potential for d.c. amperometry is the lowest which will produce a diffusion limited current. Increasing the potential beyond this value will increase only the noise and not the signal, and also decrease selectivity by allowing more species to be oxidized. Cyclic voltammetry or rotated disk voltammetry can be used to determine the optimum applied potential. Alternatively, a plot of peak height versus applied potential can be generated using d.c. amperometry

by making multiple injections of analyte and increasing the potential after each injection. A plot created using this method with serotonin as the analyte is shown in Figure 8. From this plot, the optimum applied potential for serotonin should be about 0.7V. The chromatography of serotonin plus several other neurotransmitters is shown in Figure 9. The applied potential was set to 0.8V because some of the other analytes required a higher potential.

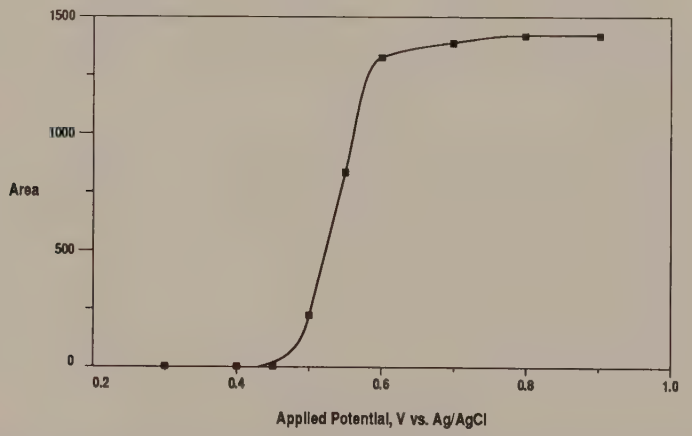


Figure 8 Peak area versus applied potential for serotonin. Peak areas were measured after making multiple injections at increasing applied potentials.

The current resulting from oxidation or reduction of analyte molecules is dependent on many factors; the most important of which is the concentration of analyte. Other factors include temperature, the surface area of the working electrode, and the linear velocity of the flowing stream over the surface of the working electrode.

Peaks (200 ng each):

1. Norepinephrine
2. Epinephrine
3. 3,4–Dihydroxybenzylamine
4. Dopamine
5. Serotonin
6. 5–Hydroxyindoleacetic Acid
7. Homovanillic Acid

Figure 9 Reversed phase separation of catecholamines using d.c. amperometric detection at a glassy carbon electrode; 0.8V versus Ag/AgCl. Column: Zorbax C-18. Mobile phase contains 5 mM triethylamine, 1 mM butanesulfonic acid, 70 mM phosphate buffer at pH 3, and a gradient of 5% to 56% methanol.

Pulsed Amperometry

The development of pulsed amperometric detection grew from the need to detect carbohydrates. Since most carbohydrates contain no UV chromophore, UV absorbance detection can only be used at very low wavelength. The detection of carbohydrates at 210 nm is insensitive and nonselective. Refractive index detection, also insensitive and nonselective, had been the most commonly used detection method. Pulsed amperometric detection is replacing these optical methods for carbohydrates, and is now being used for other nonchromophoric molecules containing alcohol, aldehyde, amine, or sulfur functional groups.

Although carbohydrates can be oxidized at gold and platinum electrodes, the products of the oxidation reaction poison the surface of the electrode, inhibiting further analyte oxidation. By repeatedly pulsing between high positive and negative potentials, a stable and active electrode surface can be maintained. Pulsed amperometric detection at a gold electrode is a reproducible and sensitive method for all carbohydrates of molecular weight up to ten-thousand.

Carbohydrates can only be detected by pulsed amperometry in high pH solutions (above pH 11). They are also very weak acids with pK_a's around

Table II
Analytes Detected by Electrochemistry

Analyte or Analyte Class	Detection Method	Working Electrode	Conditions	Ref.†
Alcohols, glycols, aldehydes	Pulsed Amp.	Pt	Mobile phase pH < 2. PED program 1.*	17,18
Amines; 1, 2, 3	Pulsed Amp.	Au	Mobile phase pH > 11. PED program 2.*	
Amines; 1, 2, 3	Int. Amp.	Au	Mobile phase pH > 11. PED program 3.*	26
Amines; 1, 2, 3, 4	Conductivity		Cation exchange with HCl/DAP mobile phase or cation ion-pair. Cation membrane suppressor.	3
Carbohydrates	Pulsed Amp.	Au	Mobile phase pH > 11. PED program 2.*	19–24
Carboxylic acids	Conductivity		Anion exchange or anion ion-pair with basic mobile phase. Anion membrane suppressor.	4, 5
Carboxylic acids	Conductivity		Ion exclusion chromatography, anion-ICE suppressor.	5
Catecholamines, phenols	d.c. Amp	Glassy Carbon	0.6–1.2V	11, 14, 15, 16
Cyanide, sulfide	d.c. Amp.	Ag	Anion exchange with mobile phase pH > 10. 0.05V	9, 10
Fatty acids	Conductivity		Reversed phase ion suppression with anion membrane suppressor.	6
Inorganic strong acid anions (Cl^-, NO_3^-, PO_4^{3-}, SO_4^{2-})	Conductivity		Anion exchange or anion ion-pair with basic mobile phase. Anion membrane suppressor.	1, 2, 4
Inorganic strong base cations (alkali metals, alkaline earths)	Conductivity		Cation exchange with HCl/DAP mobile phase or cation ion-pair. Cation membrane suppressor.	3
Iodide	d.c. Amp.	Pt	0.8V	8
Phosphates and phosphonates	Conductivity		Anion exchange or anion ion-pair with basic mobile phase. Anion membrane suppressor.	
Sulfates and sulfonates	Conductivity		Anion exchange or anion ion-pair with basic mobile phase. Anion membrane suppressor.	

Table II
Analytes Detected by Electrochemistry (continued)

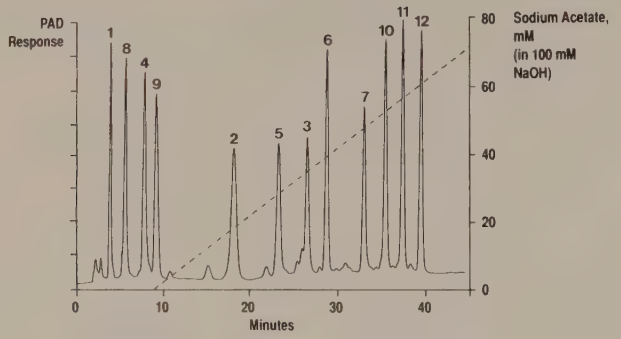
Analyte or Analyte Class	Detection Method	Working Electrode	Conditions	Ref.†
Sulfite	d.c. Amp.	Pt	Ion exclusion separation in acidic mobile phase. 0.7V.	12, 13
Sulfides, thiols, mercaptans, sufoxides	Int. Amp.	Au	PED program 4.*	25

* See Table III for PED programs
† See "Further Reading" for references

12 and are easily separated on high efficiency anion exchange columns such as Dionex CarboPac columns. Mobile phase pH's of 11 to 13 are used (1 mM to 100 mM NaOH). Sodium acetate, added to increase the strength of the mobile phase, is used to elute oligo- and polysaccharides. The combination of anion exchange separation with pulsed amperometric detection is a powerful method for the determination of carbohydrates. Although there can be some interference from amines and certain sulfur species, the technique is very sensitive and selective.

Species Detected by Pulsed Amperometry. Pulsed amperometric detection has now emerged as the most sensitive and selective method for the detection of carbohydrates. Many new applications are being developed for the determination of carbohydrates in foods and beverages, and in biological tissues and fluids. Anion exchange with pulsed amperometric detection is becoming the principle method of analyzing the carbohydrate portions of glycoproteins (Figure 10). Because of its freedom from interferences, pulsed amperometry can be used to analyze complex samples such as bacterial cell culture extracts (Figure 11).

Other species which can be detected by pulsed amperometry include alcohols, aldehydes, amines (primary, secondary and tertiary, including amino acids), and many organic sulfur species. Thiols, sulfides, and mercaptans can be detected, but fully oxidized sulfur species such as sulfates, sulfonates, and sulfones cannot. Table II lists the species most commonly determined and the conditions used. Table III lists programs which can be used on the PED for species detected by pulsed amperometry.



Column: CarboPac PA1 (4 x 250 mm) **Flow Rate: 1.0 mL/min.**

1. Fucα (1,3) GlcNAcβ (1,2) Man
2. Galβ (1,4) GlcNAcβ (1,3) Galβ (1,4) Glc
3. Galβ (1,3) GlcNAcβ (1,.3) Galβ (1,4) Glc
4. Galβ (1,4) GlcNAcβ (1,2)
   Galβ (1,4) GlcNAcβ (1,4) — Man – OH
5. Galβ (1,4) GlcNAcβ (1,2)
   Galβ (1,4) GlcNAcβ (1,4) — Man
6. Galβ (1,4) GlcNAcβ (1,6)
   Galβ (1,4) GlcNAcβ (1,3) — Manα (1,2) Man
7. Galβ (1,4) GlcNAcβ (1,2) Manα (1,6)
   Galβ (1,4) GlcNAcβ (1,2) Manα (1,3) — Man
8. Fucα (1,3) GlcNAcβ (1,2) Manα (1,6)
   Fucα (1,3) GlcNAcβ (1,2) Manα (1,3) — Man
9. Galβ (1,4) GlcNAcβ (1,2) Manα (1,6)
   Fucα (1,3)
   Galβ (1,4) GlcNAcβ (1,2) Manα (1,3) — Man
   Fucα (1,3)
10. Galβ (1,4) GlcNAcβ (1,2) Manα (1,6)
    Galβ (1,4) GlcNAcβ (1,2) Manα (1,3) — Man
    Galβ (1,4) GlcNAcβ (1,4)
11. Galβ (1,4) GlcNAcβ (1,6)
    Galβ (1,4) GlcNAcβ (1,2) Manα (1,6)
    Galβ (1,4) GlcNAcβ (1,2) Manα (1,3) — Man
12. Galβ (1,4) GlcNAcβ (1,6)
    Galβ (1,4) GlcNAcβ (1,2) Manα (1,6)
    Galβ (1,4) GlcNAcβ (1,2) Manα (1,3) — Man
    Galβ (1,4) GlcNAcβ (1,4)

Figure 10 Pulsed amperometric detection using a gold working electrode of oligosaccharides from glycoproteins. Separation was on a CarboPac PA1 with a 9 to 75 mM sodium acetate gradient in 100 mM NaOH. (Reprinted with permission from ref. 23.)

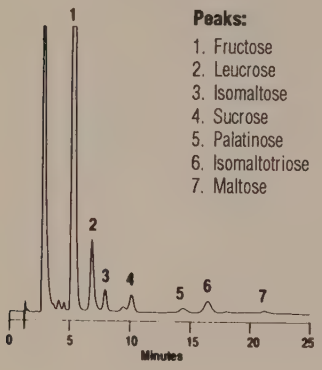


Figure 11 Carbohydrates in a bacterial cell culture using pulsed amperometric detection at a gold electrode. Separation was on a CarboPac PA1 with 150 mM NaOH.

Principles of Pulsed Amperometry. To understand the mechanism of pulsed amperometry it is first necessary to study the oxidation of an analyte using a conventional electrochemical technique such as cyclic voltammetry. The cyclic voltammetry of glucose in 0.1M sodium hydroxide using an Ag/AgCl reference electrode is shown as an example in Figure 12. The dashed line in the figure is the current resulting from the 0.1M NaOH supporting electrolyte in the absence of glucose analyte; that is, the background current. Beginning at –0.8V and sweeping in a positive direction, the background current is flat until approximately 0.25V, where oxidation of the surface of the gold electrode to gold oxide begins. Following reversal of the potential sweep direction at 0.6V, the gold oxide is reduced back to gold, with the negative peak current at 0.1V.

With glucose added to the solution, the current rises slightly as the potential is swept in a positive direction from –0.8V and remains unchanged until glucose oxidation begins. This causes the current to rise at –0.15V towards a peak at 0.26V. The current then decreases for two reasons. First, the concentration of glucose at the electrode surface has been depleted because much of it has been oxidized. Second, the formation of gold oxide inhibits further glucose oxidation. On the reverse scan, the current actually reverses from reducing to oxidizing (positive) at the onset of the gold oxide reduction. As soon as the reduction of gold oxide back to gold begins, oxidation of glucose also begins.

Table III
Pulsed Electrochemical Detector Programs
Using Pulsed and Integrated Amperometry

Analytes	Method	Working Electrode	Potentials* t (s)	E (V)	Integration Period Begin (s)	End (s)
Program 1:	Pulsed	Pt	0.00	0.20	0.20	0.40
Alcohols,	Amp.		0.40	0.20		
Glycols,			0.41	1.25		
Aldehydes			0.50	1.25		
			0.51	–0.10		
			0.60	–0.10		
Program 2:	Pulsed	Au	0.00	0.10	0.30	0.50
Carbohydrates,	Amp.		0.50	0.10		
Amines			0.51	0.60		
			0.59	0.60		
			0.60	–0.60		
			0.65	–0.60		
Program 3:	Int. Amp.	Au	0.00	–0.10	0.20	0.70
Amines			0.20	–0.10		
			0.30	0.35		
			0.40	0.35		
			0.50	–0.10		
			0.70	–0.10		
			0.71	0.90		
			0.90	0.90		
			0.91	–0.90		
			1.00	–0.90		
Program 4:	Int. Amp.	Au	0.00	–0.10	0.20	0.90
Sulfur species			0.20	–0.10		
			0.40	0.70		
			0.50	0.70		
			0.70	–0.10		
			0.90	–0.10		
			0.91	0.90		
			1.00	0.90		
			1.01	–0.90		
			1.10	–0.90		

* Ag/AgCl reference electrode. Refer to text or PED manual to convert potentials when using the pH reference electrode.

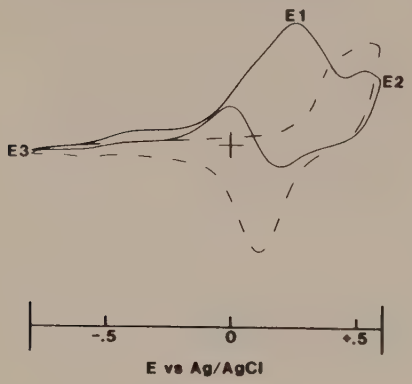


Figure 12 Cyclic voltammetry of glucose on a gold electrode. The dashed line shows current as a function of potential in the presence of 100 mM NaOH supporting electrolyte (the background). The solid line is after the addition of glucose.

If d.c. amperometric detection were used, the appropriate applied potential would be approximately 0.2V. This is the potential at which the glucose oxidation current is the highest and the background current the lowest. However, the use of a single potential results in rapidly decreasing sensitivity as an oxide layer forms and products from the oxidation reaction coat and poison the electrode surface. This problem is solved by first measuring the oxidation current at 0.1V, pulsing the potential to 0.6V and then back to –0.6V. The action of repeatedly forming and reducing the metal oxide surface layer cleans the electrode surface and maintains an active and stable surface. (0.1V is used for E1 instead of 0.2V because the background is slightly lower.) A three step program using these potentials is shown in Figure 13. The potentials are labelled E1, E2, and E3, and are applied for durations of t1, t2, and t3. The current is measured at E1 by integrating the current for a fixed time and storing the resulting charge in a sample-and-hold amplifier until the next measurement. The step from E3 back to E1 is from –0.6V to 0.1V, which charges up the electrode/solution interfacial capacitance. The carbohydrate oxidation current is integrated after a delay which allows the charging current to decay. Similar triple-potential programs are used to detect other species, especially alcohols and aldehydes (Table III).

Optimizing the Program Parameters. To find the optimum potentials and times for pulsed amperometric detection, one begins with a basic program and then optimizes the parameters. The basic program is chosen from the

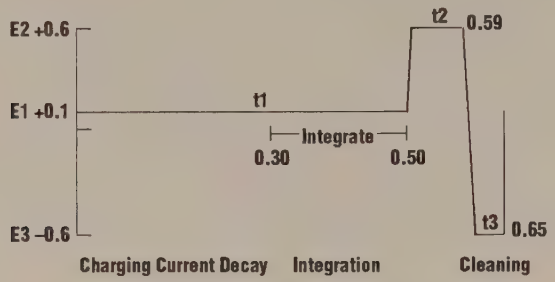


Figure 13 Recommended pulse sequence for carbohydrates. After a delay of 0.3 second to allow charging current to decay, the current is integrated for 0.2 second. t2 and t3 are the positive and negative cleaning potentials. Total waveform period is 0.65 second.

voltammogram or from published parameters for similar compounds, such as those listed in Table III. First, E1 is chosen to be the potential providing the largest ratio of analyte oxidation current to background current. A 0.5 second period for t1 containing a 0.3 second delay period and a 0.2 second integration period are good starting values. E2 and E3 are set near the positive and negative potential limits. For starting values, use 0.1 second durations for both. To optimize all of these parameters, each one must be varied independently and the effect on both signal and baseline level and noise determined. For example, the dependence of background charge on delay time for carbohydrate determinations is shown in Figure 14. From this plot it can be seen that a delay time of 0.2 second is sufficient to allow all of the charging current to decay to zero. The dependence of detector response on delay time is shown in Figure 15. This plot is developed by making repeat injections while moving the integration period to later times. Since only a minor decrease in response occurs between zero and 0.2 second, a delay time of at least 0.2 second provides the optimum ratio of signal to background. Similar plots are then created for E1 and for the positive and negative cleaning times and potentials by making repeat injections while varying their values. The cleaning potentials should be the least positive and negative potentials that produce both maximum detector response and reproducible response. To maintain an accurate reproduction of the eluting peak, a rapid sample rate is obtained using entire programs that are as short as possible. The resulting program is usually a compromise of each of these goals. For example, doubling the integration

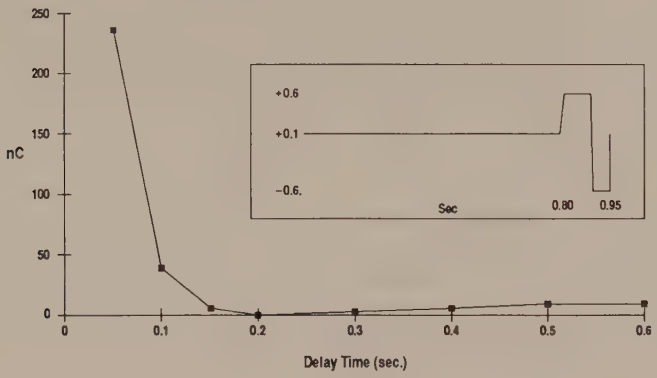


Figure 14 Background charge versus delay time using a gold working electrode with 100 mM NaOH supporting electrolyte. A 0.2 second integration period was used. The background charge decays rapidly after the pulse from E3.

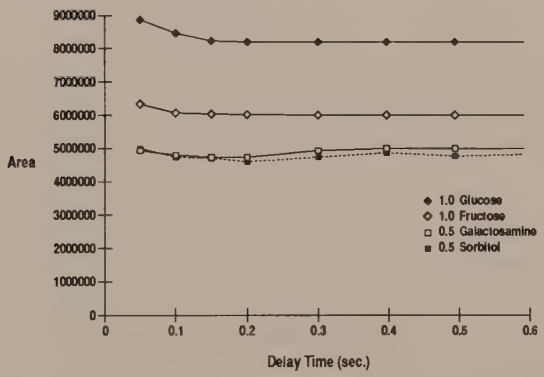


Figure 15 Peak area versus delay time for four carbohydrates using the program shown in Figure 14. Concentrations shown are in ppm. There is only a small decrease in peak area with time.

period will increase the signal-to-noise ratio, but decrease reproducibility of early eluting peaks by increasing the total potential versus time period. The program shown in Figure 13 was developed using this optimization procedure.

Current integrated for a fixed length of time is charge, and the units are coulombs. The PED reports the charge directly in coulombs. On the PAD, the charge is divided by the integration period (0.2 second) and reported in amperes. The reported current is the average current during the integration period.

Integrated Amperometry

A new variant of pulsed amperometric detection is called *integrated amperometry*. (In early publications, it was called *potential sweep pulsed coulometric detection*, or PS-PCD.) This method is useful for amines and sulfur species because their oxidation at metal electrodes is catalyzed by the formation of metal oxide. Integrated amperometry greatly minimizes background effects caused by metal oxide formation. Also, integrated amperometry decreases the effect of changes in pH, allowing moderate pH gradients to be performed without large shifts in the baseline.

The principle of integrated amperometry is illustrated in Figure 16. With pulsed amperometry, the current is integrated at a single potential following a pulse. With integrated amperometry, the current is integrated while the potential is swept across the metal oxide formation wave and also during the reverse sweep across the oxide reduction wave. Without the

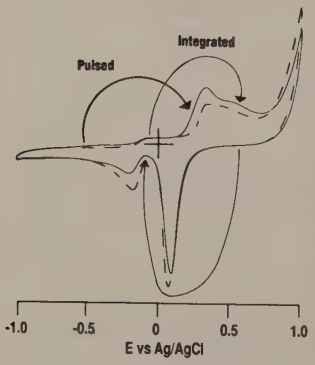


Figure 16 Comparison of pulsed and integrated amperometry for the amino acid leucine. Cyclic voltammetry is on a gold working electrode in 100 mM NaOH. In pulsed amperometry, current is measured after a pulse and a short delay to allow charging current to decay. In integrated amperometry, current is integrated continuously during a cycle where the electrode is oxidized and then reduced back to its original state. Integrated amperometry provides a more stable baseline and a larger signal for species catalytically oxidized by the metal working electrode's oxide layer.

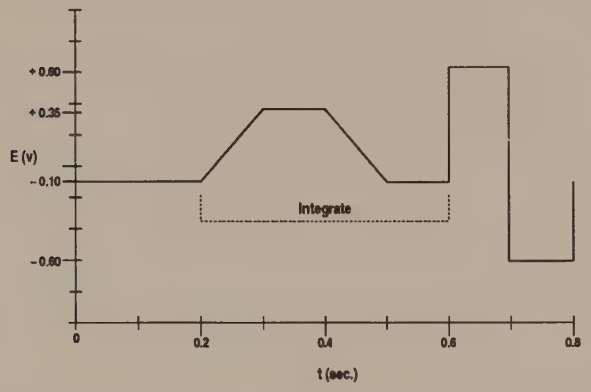


Figure 17 Example integrated amperometry program for amines detected on a gold electrode with an Ag/AgCl reference electrode. Following the integration cycle, short positive and negative pulses are used to clean the electrode.

presence of analyte molecules, the net charge is approximately zero. Positive and negative cleaning pulses are added to the potential versus time program following the integration period. An example program is shown in Figure 17. Programs are optimized using a similar technique to the method used for pulsed amperometry.

The advantage of integrated amperometry is that by cancelling the charge from oxide formation and reduction, the effect on the baseline is greatly minimized. Also, the total current is less sensitive to the changes in oxide formation and reduction potentials caused by changes in pH. The potentials at which the surface of metal electrodes are oxidized shift negative as pH increases. Integrated amperometry can be used to minimize the effect of these changes by setting the limits of the potential sweep to potentials where little change is taking place. An example of the effect of cancelling the working electrode oxidation current is shown in Figure 18. When the current is integrated only during the forward step across the metal oxide formation wave, The baseline shift during the gradient is very large (18a). Also, the eluting analytes cause dips instead of peaks because their effect on surface oxide suppression is greater than the detector response from the analytes. Integrating the current for both the forward and

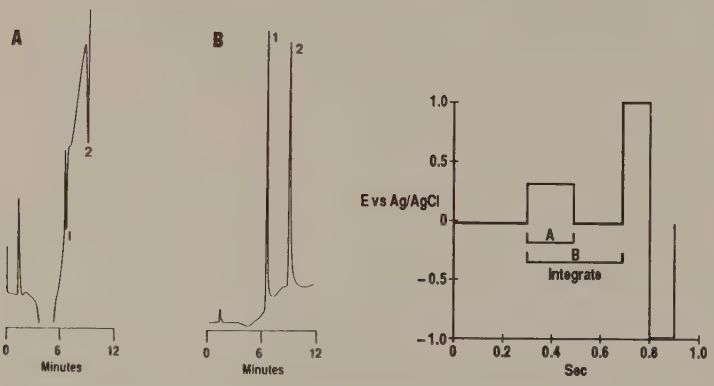


Figure 18 Comparison of two integration periods for the chromatography of 10 ppm leucine (peak 1) and lactose (peak 2) using a 15 to 150 mM NaOH gradient. In chromatogram A, cell current was integrated only during the forward pulse to 0.3V. The result is a badly shifting baseline and dips instead of peaks. Integrating both the forward and reverse pulses largely cancels background effects and greatly improves the chromatogram (B).

reverse steps greatly minimizes the baseline shift and eliminates the dips, resulting in good peaks (18b).

If the standard Ag/AgCl reference electrode is replaced with a pH electrode (described below), the effects of mobile phase pH changes can be minimized further.

Integrated amperometry is a new technique developed in Professor Dennis Johnson's laboratory at Iowa State University, and there were few applications which were developed when this book was written. However, Dr. Johnson has published several recent papers showing the utility of integrated amperometry. (These papers are listed in the "Further Reading" section.) With continuing research, further development of integrated amperometry as a useful and important means of determining amines, sulfur species, and many other organic analytes is expected. We at Dionex hope those of you reading this book will contribute to the development of integrated amperometry.

The Amperometry Cell

The PED amperometry cell is a miniature flow-through three electrode voltammetry cell with a working electrode, reference electrode and counterelectrode. Oxidation or reduction of analyte molecules is accomplished by applying a potential between the working and reference electrodes. The reference electrode is chosen such that the potential difference between it and the solution is fixed by an electrochemical redox couple. The PED amperometry reference electrode uses either a silver/silver chloride or pH reference electrode. Any changes in the potential applied between the working and reference electrode will be developed entirely between the working electrode (where analyte reduction or oxidation takes place) and the solution. To maintain a constant potential difference between the reference electrode and the solution, the cell current must be prevented from flowing through the reference electrode. A section of the PED electronic circuit (the *potentiostat*) causes the cell current to flow instead through the counterelectrode. The potentiostat automatically compensates for the solution resistance between the reference electrode and the counterelectrode.

The PED amperometry cell is a thin-layer design. The mobile phase flows in a thin channel parallel to the surface of a flat disk electrode. The resulting smooth flow produces low noise. The low volume of the thin layer channel also allows high efficiency columns to be used. The cell is designed so that the electrical resistance between the working electrode and the counterelectrode is as low as possible. This results in a wide linear dynamic range. Low resistance is accomplished by locating the counterelectrode (the stainless steel cell body) directly across the thin-layer channel from the working electrode.

Reference Electrodes

Two different reference electrodes are used with the PED: a standard silver/silver chloride (Ag/AgCl) reference electrode and a combination pH electrode. The purpose of the pH reference is to minimize changes in the baseline during a pH gradient. The potentials at which many redox reactions take place are pH dependent, with the potential shifting –0.059V

per pH unit. This is true for metal oxide formation and reduction reactions. Since the reference potential of the pH electrode also shifts by –0.059V per pH unit, pH dependent potential shifts at the working electrode are cancelled.

At an eluant pH of 7, the reference potential of the pH electrode is the same as that of the Ag/AgCl electrode. As the eluant pH is increased, the reference potential decreases approximately 0.059 volts per pH unit. For example, at an eluant pH of 12, the reference potential of the pH electrode would be –0.295V relative to the Ag/AgCl reference electrode. Therefore, at pH 12, the potentials applied to the working electrode must be raised approximately 0.3V when switching from the Ag/AgCl reference to the pH reference. In acidic mobile phases, the reference potential of the pH electrode is positive with respect to the Ag/AgCl reference, and all applied potentials must be decreased by 0.059V per pH unit when switching from the Ag/AgCl reference to the pH reference.

Working Electrodes

The PED can be used with four working electrode materials. These are: gold, silver, platinum, glassy carbon. The precious metal electrodes are very high purity solid metals. Glassy carbon is a hard graphitic substance. The analytes determined using these working electrodes are listed in Table II.

The choice of working electrode material for a given application is dependent on three factors:

- *Potential limits for the working electrode in the mobile phase*
- *Involvement of the electrode in the electrochemical reaction*
- *Kinetics of the electron transfer reaction*

Potential Limits. The negative potential limit is the potential at which the mobile phase or supporting electrolyte is reduced. At the positive potential limit, the mobile phase, the supporting electrolyte, or the electrode itself can be oxidized. Because these reactions will produce current far in excess of the analytical redox reaction, the potential used to detect the analyte

must be within these limits. Table IV lists the potential limits for the four electrode materials in acidic and basic solutions. The potential limits are strongly affected by the pH of the mobile phase. Negative potential limits are more negative in base and more positive in acid. Conversely, positive limits are more positive in acid and more negative in base. In other words, the usable potential window shifts negative in basic solutions and positive in acidic solutions.

As the applied potential approaches the potential limit, the noise will increase as the background current increases. On metal electrodes, there is a sharp increase in background current as the potential limit is exceeded. On glassy carbon, the increase in background current is more gradual. Because the maximum applied potential that can be used is determined by the required signal-to-noise ratio, the set of values listed in Table IV is only a rough guide. For some applications using glassy carbon, it may be necessary to exceed these limits.

Table IV
Potential Limits in Acidic and Basic Solutions vs. Ag/AgCl Reference Electrode*

Working Electrode	Solution (0.1 N)	Negative Limit (Volts)	Positive Limit (Volts)
Glassy Carbon (GC)**	KOH	(–1.5)	(+0.6)
	$HClO_4$	(–0.8)	(+1.3)
Gold (Au)	KOH	–1.25	+0.75
	$HClO_4$	–0.35	+1.10
Silver (Ag)	KOH	–1.20	+0.10
	$HClO_4$	–0.55	+0.40
Platinum (Pt)	KOH	–0.90	+0.65
	$HClO_4$	–0.20	+1.30

* These are approximate values. Potential limits in neutral solutions will be intermediate.

** Unlike metallic electrodes, the potential limits for the glassy carbon electrode do not cut off sharply. The noise and background level will differ from application to application and must be determined experimentally.

The largest positive potential limits are obtained on glassy carbon and platinum. Accordingly, oxidations are often performed using one of these two materials. The largest negative potential limits, listed in order, are obtained on glassy carbon, silver and gold. Because of the ease of reducing hydrogen ion to hydrogen gas on a platinum electrode, platinum has a poor negative potential limit and is generally not used for reductions.

When potentials are used that are more negative than approximately –0.1V versus an Ag/AgCl reference electrode, high background current is caused by the reduction of molecular oxygen dissolved in the mobile phase. This background current can be greatly reduced by degassing the mobile phase.

Involvement of the Electrode in the Redox Reaction. The reaction mechanism for the oxidation of many analytes is the transfer of electrons from the analyte molecules to the electrode. The electrode acts as an inert electron sink and is otherwise not involved in the oxidation reaction. When this is the reaction mechanism, carbon is often the prefered electrode material. Examples include the detection of catecholamines or nitrite. In contrast, silver and gold can be oxidized in the presence of complex or precipitate-forming ions. For the detection of these ions, the working-electrode material is directly involved in the reaction and is actually slowly consumed, although so slowly that no degradation in performance is observed. For example, silver can be oxidized to silver cyanide in the presence of cyanide ion. This reaction takes place at a much lower potential than the oxidation of cyanide to cyanate at a platinum electrode. The ability to use a lower applied potential increases the selectivity of the analysis, as fewer other species will be oxidized. Also, noise caused by the oxidation of trace contaminants in the mobile phase will be decreased at the lower applied potential. A silver electrode can also be used to detect other complex or precipitate-forming ions. These include bromide and iodide, as well as numerous sulfur containing species such as sulfide, sulfite, thiosulfate, and organic thiols. One disadvantage to the use of silver or gold for oxidations is that the presence of halides in the mobile phase will greatly decrease the positive potential limit. Halides can usually be replaced by nonreacting anions such as acetate, perchlorate, nitrate, phosphate, or sulfate.

Kinetics of the Electron-Transfer Reaction. For a kinetically fast electron-transfer reaction, the ratio of the oxidized to the reduced form of the analyte species will be described by the Nernst equation (page 22). If the reaction rate is fast, then the reaction is said to be reversible. Note that if E_{app} is set 0.118V positive of E°, then (for n = 1) the ratio of the oxidized to the reduced form of the analyte at the electrode surface will be 100:1. Nearly all of the analyte that reaches the electrode will be oxidized. The limiting current will have been attained, and no advantage will be gained from a further increase in the applied potential. For a slow electron-transfer reaction, a potential considerably in excess of E° is required to drive the reaction at a fast rate. This excess voltage is called overpotential, and the reaction is said to be irreversible. The more irreversible the redox reaction, the greater the applied potential must be. This will result in more noise and less selectivity. The oxidation or reduction of many species is more facile on one electrode material than on another. This is particularly true for small inorganic species, many of which can be oxidized or reduced much more easily on platinum than on carbon. For example, platinum electrodes are used to detect arsenite, iodide and sulfite.

A major consideration when choosing an electrode material is its ability to maintain an active surface. Electrodes will develop a layer of surface oxide at positive applied potentials. This buildup will inhibit the oxidation of the analyte, often resulting in a decreasing response with repeated injections. The active surface can be renewed by polishing the electrode. Glassy carbon electrodes are more resistant to poisoning by oxide formation than are metallic electrodes, and do not need to be polished as often. It is for this reason that glassy carbon is used far more extensively than any other electrode material for d.c. amperometry. Pulsed amperometry and integrated amperometry solve this problem for platinum and gold electrodes by pulsing to high positive and negative potentials after each current measurement.

Conclusion

Several forms of electrochemical detection have become popular for certain HPLC applications. d.c. amperometry is an accepted method for neurochemical analyses. Conductivity is the workhorse detection method in ion chromatography, just as UV detection is for HPLC. Pulsed amperometry is now emerging as the superior detection method for carbohydrates. However, electrochemical detection has never caught on as a routinely used method with power rivaling UV-visible absorbance detection. This is partly because UV-visible detectors were developed first, and partly because electrochemical detectors are perceived to be harder to use, requiring more operator education. On the other hand, attempting to detect all species by UV-visible absorbance can be severely restricting. For most of the numerous analytes listed in the Introduction, detection by UV-visible absorbance is poor, while one of the three main techniques of electrochemical detection discussed in this article provides superior sensitivity and selectivity.

Electrochemical detection is not a substitute for UV-visible absorbance detection, but is instead an important complement. A liquid chromatograph equipped with both a UV-visible detector and a Pulsed Electrochemical Detector is a truly versatile analytical instrument.

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